2005

Research Update Meeting 2005 - Upright Dieback 2005

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Upright dieback

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Quick Review – Upright Dieback Symptoms

- Tip dieback

- “Salt and pepper” pattern
- Estimated 20-25% affected in severe cases

Upright Dieback: Not just dead uprights

“Upright dieback” does *not* include

- Abiotic factors
  - drought, winter, and chemical injury
- Other diseases
  - fairy ring, Phytophthora root rot
- Rodent damage

Causal agent(s) ?

Frequently isolated from diseased uprights:

- *Aureobasidium pullulans*
- *Colletotrichum acutatum* *
- *Colletotrichum gloeosporiodes* *
- *Fusicoccum putrefaciens* *
- *Gloeosporium sp.*
- *Pestalotia sp.*
- *Phomopsis vaccinii* *
- *Synchronoblastia crypta*

* Cause field/storage rot

Causal agent(s) ?

- Previously only *Synchronoblastia crypta* proven to be a pathogen

- *Phomopsis vaccinii* suspected
  - Frequent recovery
  - Causes tip dieback/canker diseases of blueberry

Objectives

- Determine role of *Phomopsis vaccinii* in upright dieback

  1. Prove pathogenicity
  2. Determine sites of infection
  3. Determine location of fungus in plant tissue

Objective 1:

Prove pathogenicity of *Phomopsis*

To determine role of *Phomopsis vaccinii* in upright dieback, Koch's Postulates must be completed

- **Koch's Postulates** – to prove pathogenicity
  1. **Isolate pathogen** routinely from diseased plants
  2. **Obtain pure culture** of suspected pathogen
  3. **Inoculate** healthy plant with suspected pathogen
  4. **Observe symptoms** that are the same on the inoculated plant as the symptoms in the field
  5. **Re-isolate** pathogen

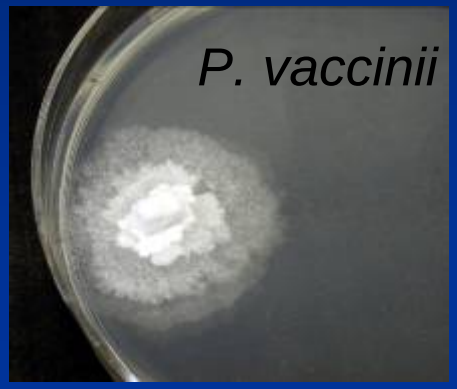
- **Why this is important:**
  - Easier to identify and study disease

Inoculations

- Inoculations conducted with
  - Tissue culture plants in lab
  - Rooted cuttings in greenhouse
  - Cultivars
    - Early Black
    - Stevens

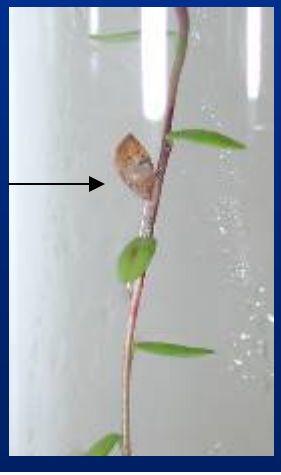
General inoculation procedure

- Small plug of agar with fungus placed on plant



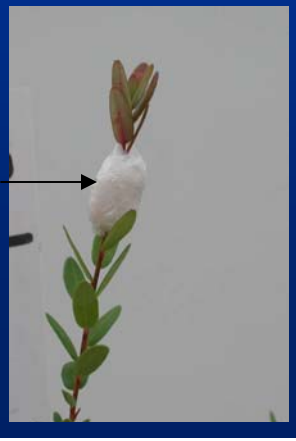
Tissue-cultured plants

inoculum



Rooted cuttings

inoculum



Inoculations – Results

- Koch's postulates **completed** for *Phomopsis vaccinii*
  - Indicating that *Phomopsis vaccinii* is a causal agent of upright dieback disease

P. vaccinii dieback – Tissue Culture

Dieback

Healthy

P. vaccinii dieback – Rooted Cuttings

Phomopsis Isolate Tests

- Previously, all *Phomopsis* isolated from cranberries and blueberries were thought to be *Phomopsis vaccinii*

- But recent evidence indicates that there are numerous species of *Phomopsis* isolated from cranberry and blueberry

Phomopsis Isolate Tests

- Different isolates tested for pathogenicity
  - *Phomopsis vaccinii*
    - Isolates from
      - Cranberry stems (3)
      - Blueberry fruit (1)
      - Blueberry stem (1)
  - *Phomopsis* sp.
    - Isolates from
      - Cranberry stems (2)
      - Blueberry stems (5)
- Test conducted on
  - Tissue culture plants (cv. Early Black and Stevens)
  - Rooted cuttings in greenhouse (cv. Early Black)

Phomopsis Isolate Tests – Results

- Both *Phomopsis vaccinii* and *Phomopsis* sp. caused upright dieback symptoms
- Isolates from both blueberry and cranberry caused symptoms
- But not all of the isolates tested in any category caused symptoms
  - Category ⇒
    - *Phomopsis vaccinii* isolates
    - *Phomopsis* sp. isolates
    - Cranberry isolates
    - Blueberry isolates

Objective 2:

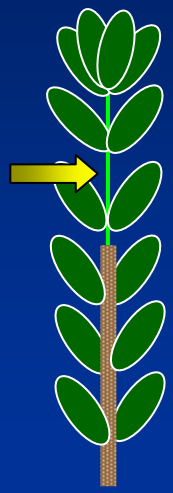
Determine sites of infection

Infection Site – Inoculation Procedure

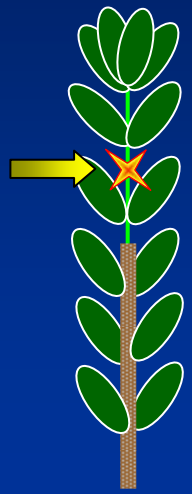
- Inoculation procedure similar to previously described
- Two regions of uprights were inoculated
  - New growth
    - green herbaceous tissue of the current year's growth
  - Old growth
    - woody tissue of past-year's growth
- Different wounding methods

Infection Site Inoculation – Wounding methods

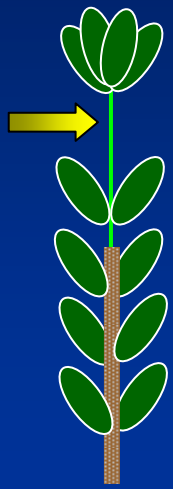
New Growth



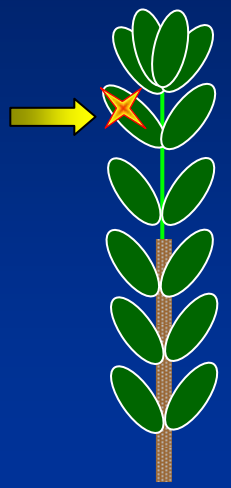
no wound



stem pierce

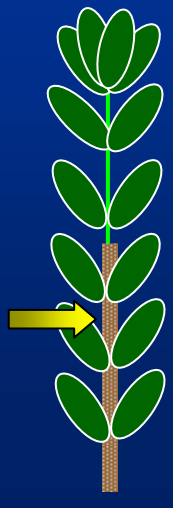


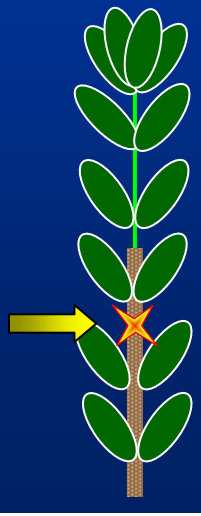
leaf removal

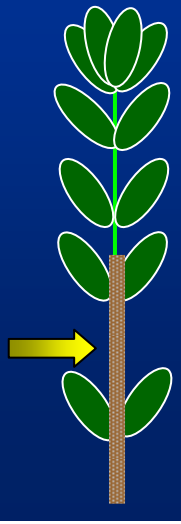


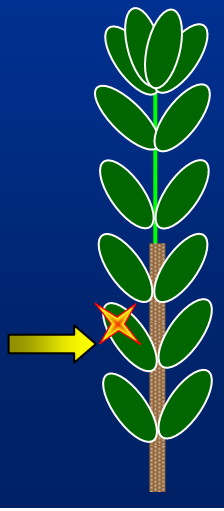
leaf pierce *

Old Growth









* Trial 2 only

Infection Site – Results

- **First Trial**
  - Symptoms
    - Only new growth
      - Only wounded plants
- **Second Trial**
  - Unlike 1st trial, symptoms also observed on:
    - New growth
      - Non-wounded plants
    - Old growth
      - Wounded

Symptom development differed for various wounding methods

TRIAL 1

New growth
- stem pierce
- leaf removal

TRIAL 2

New growth
- stem pierce

TRIAL 2

New growth
- not wounded
- leaf removal
- leaf pierce

TRIAL 2

Old growth
- stem pierce
- leaf removal

Infection Site – Summary

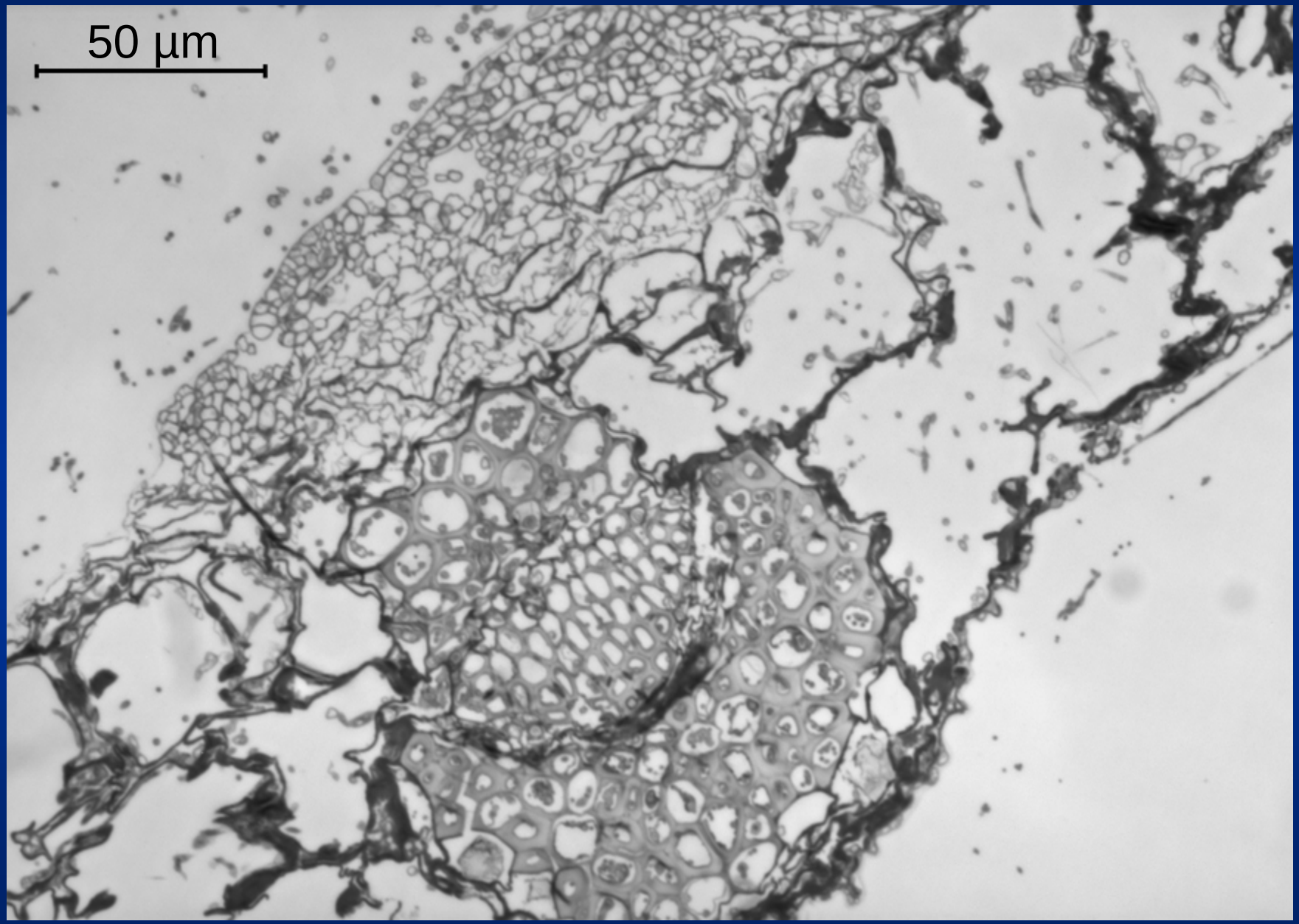
- New growth most susceptible
- Older growth susceptible to infection when wounded
- Wounded tissue more likely to be infected than non-wounded
- Only new growth is affected when infection occurs in the new growth
  - and infection does not progress to adjacent runners or uprights if the infection occurs in the old growth

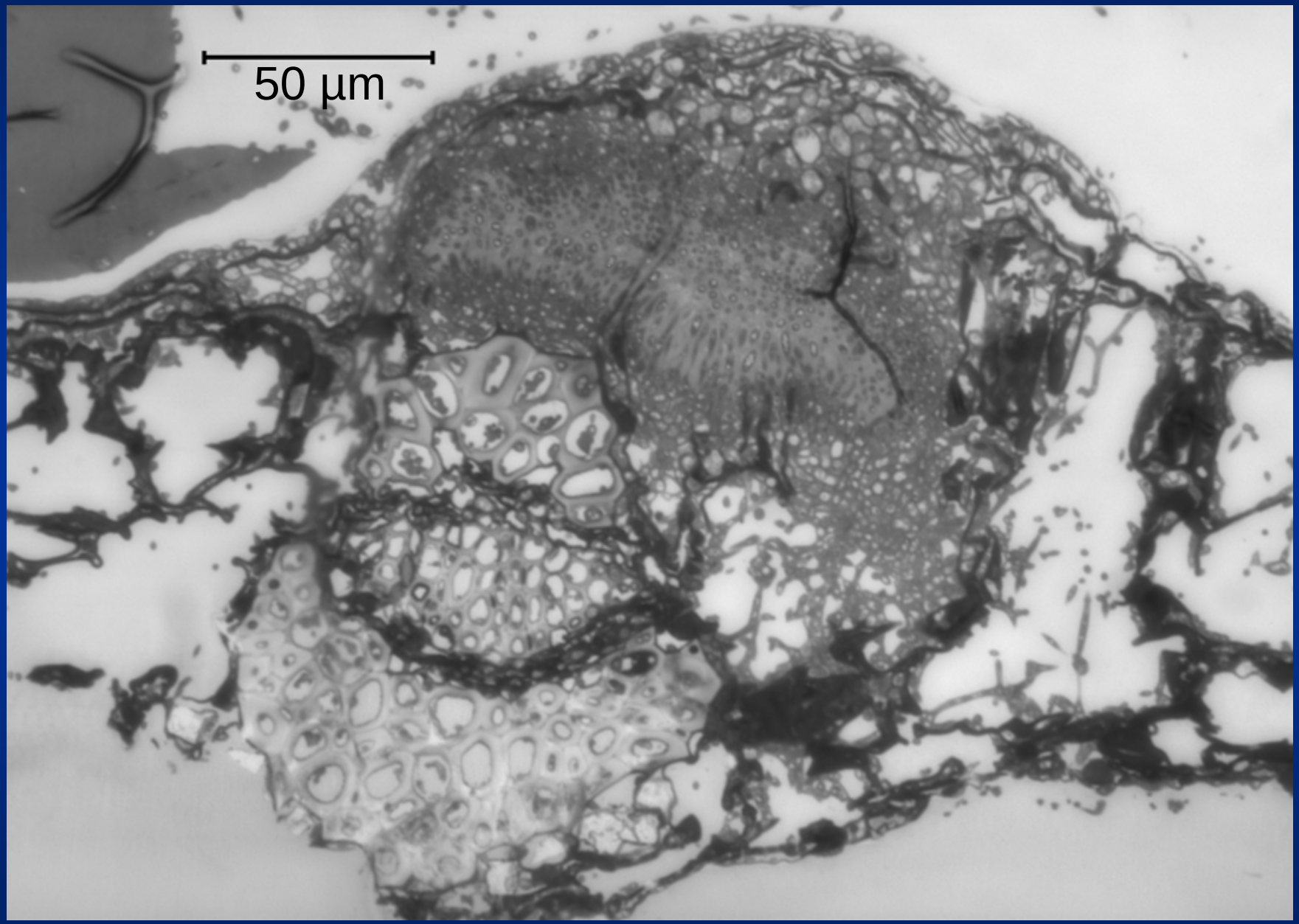
Objective 3:

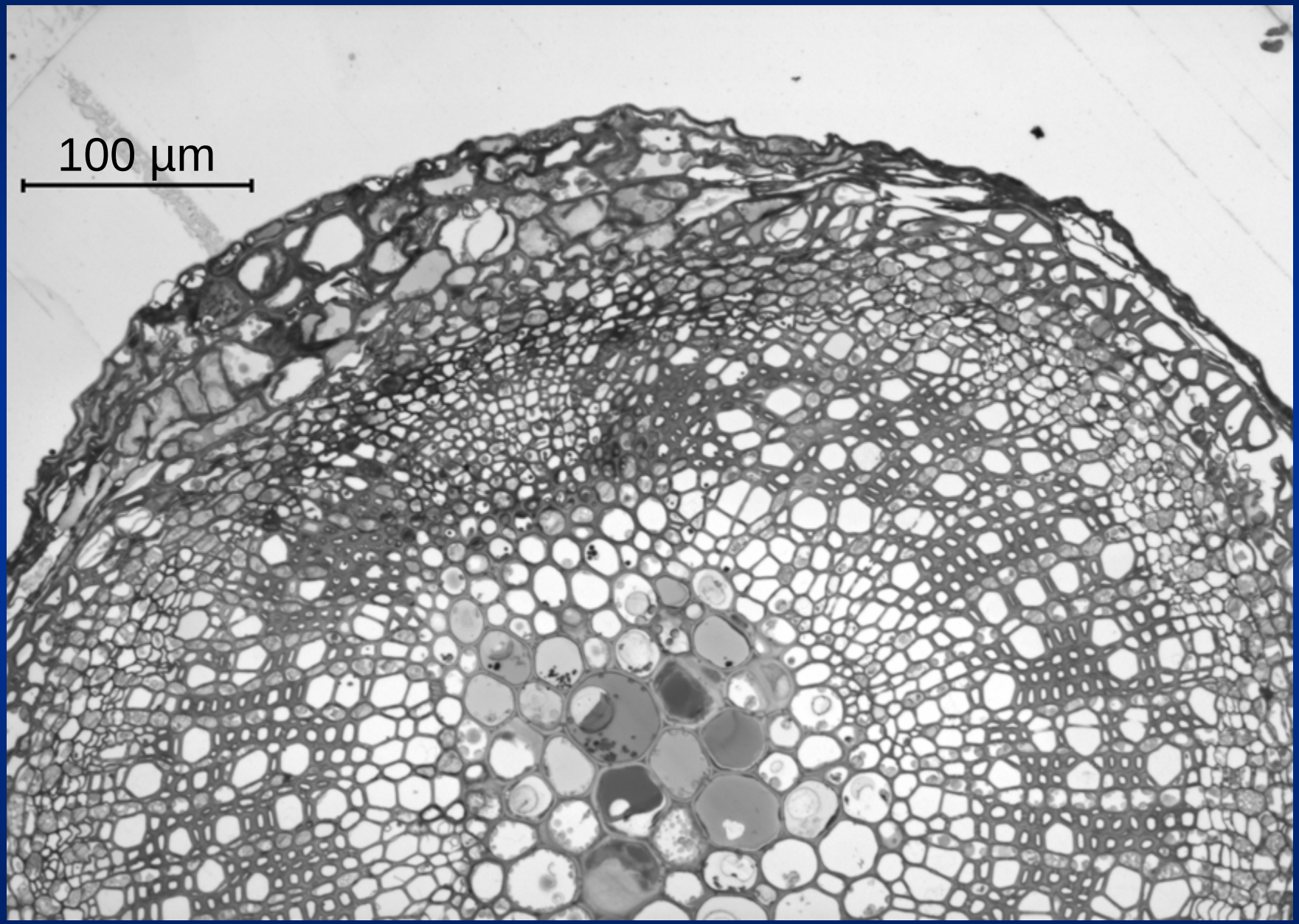
Determine location of fungus in plant tissue

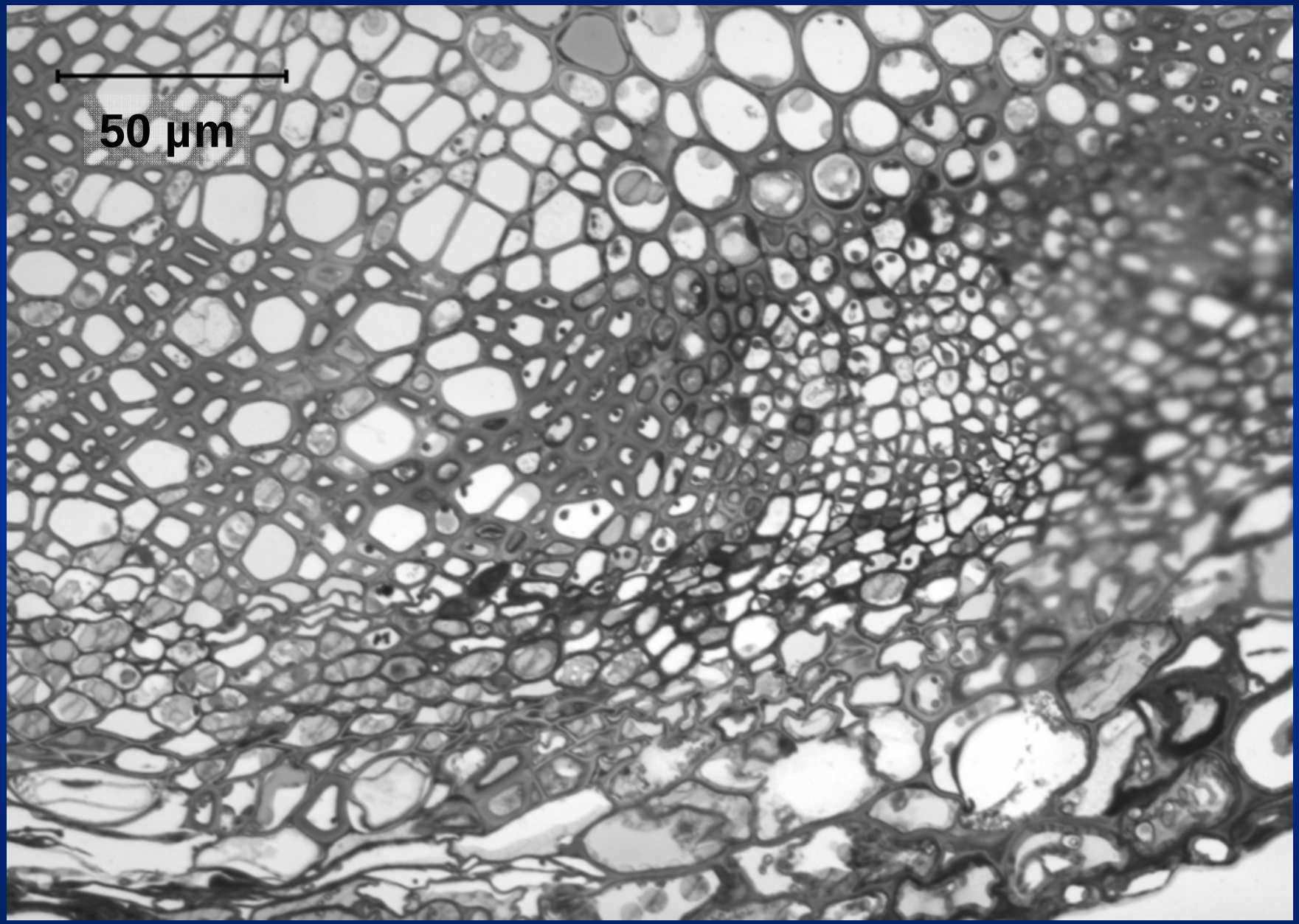
Microscopy

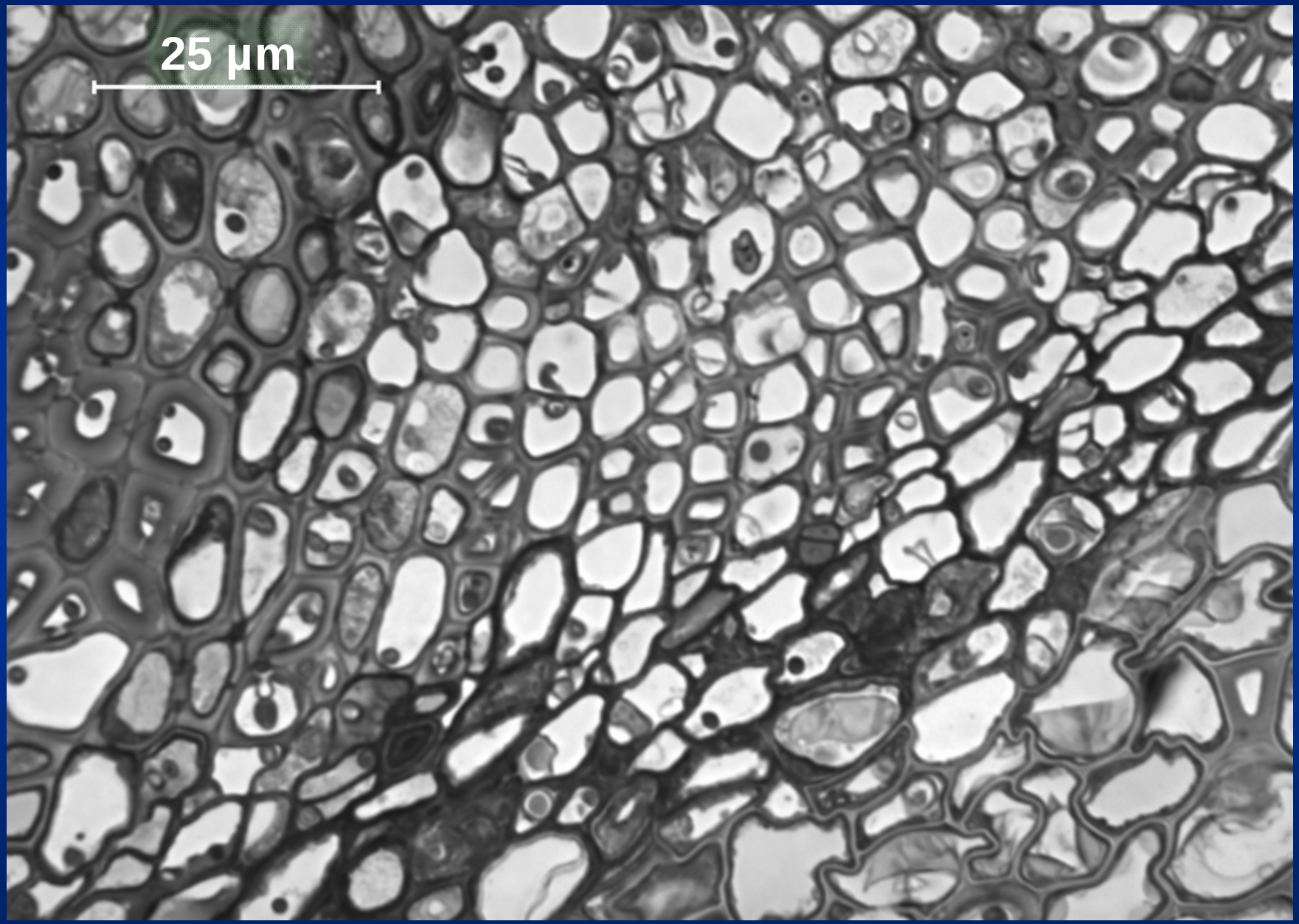
- *P.vaccinii*-inoculated tissue culture plants
  - Leaves and stems
  - Fixed and embedded
  - 1 µm sections
  - Toluidine Blue stain


50 µm


50 µm


100 µm


50 µm


25 µm

Microscope – Summary

- *P. vaccinii* observed
  - Throughout dead leaf cells
  - Vascular tissue of leaf and stem
- Host response observed

Summary of Conclusions

- **Inoculations and Isolate Tests**
  - *Phomopsis vaccinii* is a causal agent of upright dieback of cranberry
  - Isolates from both blueberry and cranberry of *Phomopsis vaccinii* and *Phomopsis* sp. can cause upright dieback

Summary of Conclusions, cont.

- **Infection Site**
  - New growth is more susceptible to infection than old growth
  - Wounded tissue more likely to be infected
  - Old growth is susceptible to infection only when wounded
  - Only new growth is affected when infection occurs in the new growth, and infection does not progress to adjacent runners or uprights if the infection occurs in the old growth

Summary of Conclusions, cont.

- **Microscopy**
  - *Phomopsis vaccinii* is a vascular pathogen

Acknowledgements

- USDA/CSREES Special Grant
- Cape Cod Cranberry Grower's Association
- Rod Serres
  - Ocean Spray Cranberries
- Dale Callaham
  - The Central Microscopy Facility at UMASS


Questions ?